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HTRA1 variant increases risk to neovascular age-related macular degeneration in Chinese population

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Abstract

Age-related macular degeneration (AMD) is a leading cause of irreversible visual impairment in the world. Advanced AMD can be divided into wet AMD (choroidal neovascularization) and dry AMD (geographic atrophy, GA). Drusen is characterized by deposits in the macula without visual loss and is an early AMD sign in the Caucasian population. rs11200638 in the promoter of *HTRA1* has recently been shown to increases the risk for wet AMD in both Caucasian and Hong Kong Chinese populations. In order to replicate these results in a different cohort, we genotyped rs11200638 for 164 Chinese patients (90 wet AMD and 74 drusen) and 106 normal controls in a Han Mainland Chinese cohort. The genotypes were compared using chi square analysis for an additive allelic model. rs11200638 was significantly associated with wet AMD ($p = 5.00 \times 10^{-12}$). Unlike in the Caucasian population, the risk allele of rs11200638 was not associated with drusen in our Chinese population. These findings confirm the association of *HTRA1* with wet AMD.

Keywords: AMD; *HTRA1*; Chinese

1. Introduction

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness affecting millions worldwide. AMD can be classified as either early or advanced. Signs of early AMD include drusen and pigmentary changes in RPE without visual loss. There are two types of advanced AMD, geographic atrophy of RPE and overlying photoreceptors (GA) or choroidal neovascularization (wet AMD). Both GA and wet AMD are associated with vision loss. Drusen is comprised of small yellowish, extracellular deposits of lipid, protein and cellular debris; accumulated hyaline excrescences in Bruch's membrane. The protein and lipid material found in drusen includes complement components and modulators (Anderson et al., 2002; Crabb et al., 2002; Hageman et al., 2005, 2001; Johnson, Leitner, Staples, & Anderson, 2001; Mullins, Aptsiauri, & Hageman, 2001; Mullins, Russell, Anderson, & Hageman, 2000) and HTRA1 (Yang et al., 2006). Despite the prevalence of AMD, the etiology of the disease is elusive. A variant rs11200638 in the promoter of *HTRA1* was found to be associated with both GA and wet AMD (Cameron et al., 2007; Dewan et al., 2006; Yang et al., 2006). The risk

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allele A of rs11200638 has been shown to increase *HTRA1* expression (Yang et al., 2006). In this paper, we investigated the association of HTRA1 and wet AMD in an independent Chinese cohort. We demonstrate that rs11200638 confers similar risk to wet AMD as was previously reported in a Hong Kong Chinese population (Dewan et al., 2006).

2. Methods

2.1. Patients

This study was approved by the Institutional Review Boards of the Sichuan Provincial People's Hospital and the University of Utah. All subjects provided informed consent prior to participation in the study. AMD patients were recruited in ophthalmology clinic at Sichuan Provincial People's Hospital. Normal age-matched controls included individuals with a normal eye examination (individuals age 60 years or older with no drusen or RPE changes). All participants went through a standard examination protocol as described previously (Yang et al., 2006). Grading was carried out using a standard grid classification suggested by the age-related Eye Disease Study Research Group AREDS (2001). The Chinese drusen cohort had drusen that were typically small (<64 μM, discrete, and non-confluent, see Fig. 1 as an example).

2.2. Genotyping

The Chinese cohort of 164 AMD patients including 74 drusen (Fig. 1b) and 90 wet AMD (Fig. 1d) was genotyped. Lab personnel blinded to case/control status compared allele frequencies to 106 age and ethnicity matched normal controls. rs11200638 and rs10490924 were genotyped as previously described (Yang et al., 2006).

2.3. Analysis for 10q26.13

The chi-squared test for trend for the additive model over alleles was performed to assess evidence for association. Odds Ratios and 95% confidence intervals were also calculated to estimate risk size for the heterozygotes and homozygotes for the risk alleles using logistical regression (SPSS v13.0). Linkage disequilibrium (LD) and Hardy–Weinberg equilibrium

Table 1
Characteristics of AMD cases and controls matched for age and ethnicity

	Total number	Male/female	Average age
Cases CNV	90	41/49	64 ± 6.6
Cases drusen	74	33/41	68 ± 7.1
Controls	106	48/58	64 ± 5.9

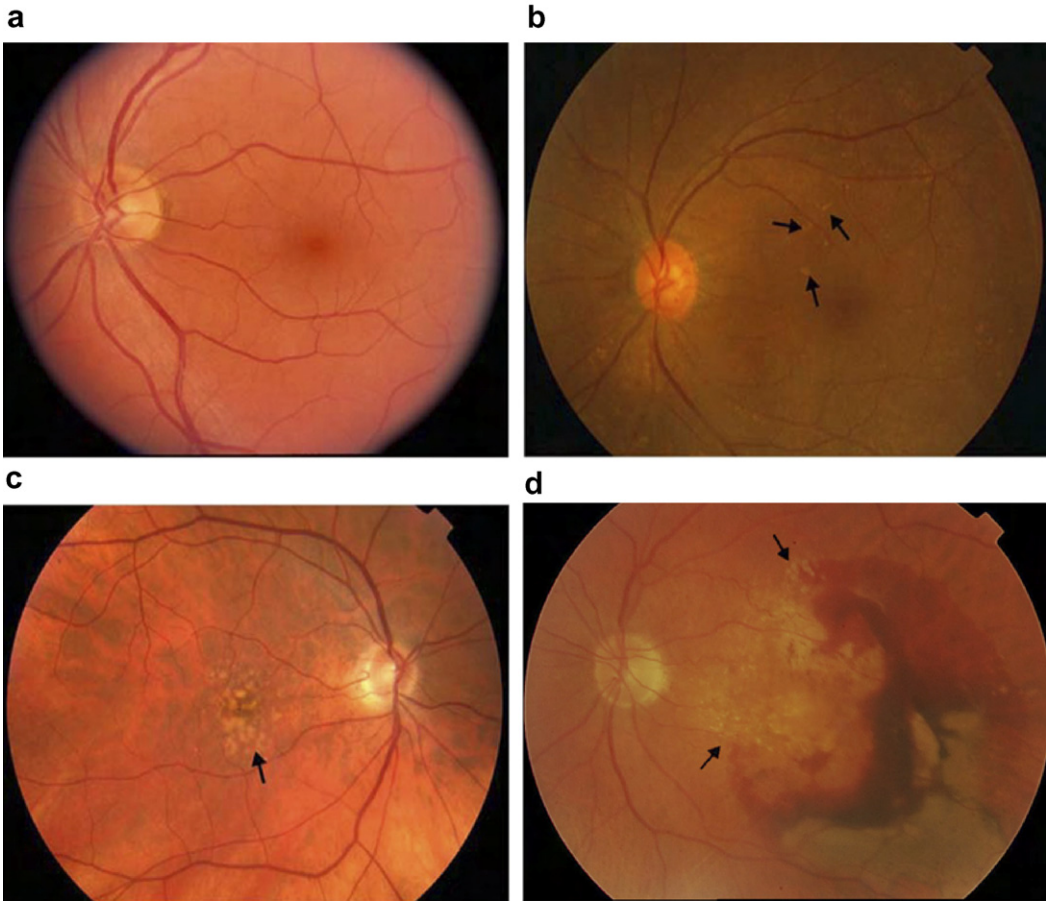

Fig. 1. Clinical features of AMD in Chinese. (a) Fundus photograph of normal eye. (b) Fundus photograph of a Chinese eye with small drusen (arrows). Note that these drusen are small in caliber (<64 μm) as compared to soft large drusen (>124 μm) in Caucasian population (arrows) (c). (d) Fundus photograph of left eye with wet AMD, note choroidal neovascular membrane (arrows) and no drusen.

Table 2
Association between subphenotypes of AMD and HTRA1 variant (rs11200638) and *LOC387715* rs10490924 in a Chinese cohort

	Phenotype	*N* (genotype count)	Risk allele frequency (%)	*p*-value	OR_{hom} (95% CI)	OR_{het} (95% CI)	PAR
Association of HTRA1 (rs11200638) and ARMS2 (rs10490924) with CNV and not drusen							
HTRA1 (rs11200638)	CNV	90 (AA:53; AG:34; GG:3)	77.78	5.00×10^{-12}	32.98 (8.80, 123.63)	5.04 (1.43, 17.79)	67.4
	Drusen	74 (AA:15; AG:43; GG:16)	49.33	0.258			
	Control	106 (AA:15; AG:63; GG:28)	43.87				
LOC387715 (rs10490924)	CNV	90 (TT:52; TG:35; GG:3)	77.22	6.83×10^{-12}	33.51 (8.95, 125.47)	5.46 (1.55, 19.22)	66.84
	Drusen	74 (TT:13; TG:45; GG:16)	47.97	0.337			
	Control	106 (TT:15; TG:62; GG:29)	43.40				

Shown are calculations for the A allele of the *HTRA1* variant (rs11200638) and the T allele *LOC387715* rs10490924 and the corresponding number of unrelated affected individuals (*N*) and genotype count, the allelic frequency in affected individuals, *p*-value, odds ratio (OR) assuming an additive model. The control group was age-matched to the patient group and the individuals had normal eye examinations.

(HWE) were examined using Haploview v3.32. No significant deviations from HWE were detected. For risk genotypes, we calculated population attributable risks (PAR) using the Levin formula (Levin, 1953).

3. Results

Recently, the A allele in SNP rs11200638 in the promoter of HTRA1 was found to be associated with the wet AMD phenotype in Caucasian (Yang et al., 2006) and Hong Kong Chinese (Dewan et al., 2006) populations. To investigate the association of HTRA1 rs11200638 with different phenotypes (drusen and Wet AMD) (Fig. 1) in a different AMD cohort, we genotyped a Mainland Chinese AMD cohort (90 wet AMD, 74 drusen) and 106 age and ethnicity matched controls. The clinical and demographic features of this Chinese cohort are listed in Table 1. We analyzed two SNPs, rs10490924 and rs11200638, which were shown to be in nearly complete LD ($D' = .98$) with each other in the Hong Kong Chinese population and significantly associated with a wet AMD phenotype (Dewan et al., 2006). rs11200638 was significantly associated with wet AMD in our cohort ($p = 5.00 \times 10^{-12}$ for additive model, $OR_{het} = 5.04$ (1.43, 17.79), $OR_{hom} = 32.98$ (8.80, 123.63); A allele: 77.78% in cases versus 43.87% in controls). We performed a separate association analysis for *LOC387715* rs10490924. Significant association was found for *LOC387715* rs10490924 ($p = 6.83 \times 10^{-12}$ for additive model, $OR_{het} = 5.46$ (1.55, 19.22), $OR_{hom} = 33.51$ (8.95, 125.47); T allele: 77.22% in cases versus 43.40% in controls). The pairwise LD analysis between *HTRA1* rs11200638 and *LOC387715* rs10490924 was performed using the HapView program and was found to be 0.99, Furthermore, we found that drusen was not associated with either *HTRA*1 rs11200638 risk allele ($p = 0.258$) or *LOC387715* rs10490924 ($p = 0.337$) (Table 2).

4. Discussion

Several independent association studies have implicated a major locus for AMD at chromosome 10q26. While rs10490924 in LOC387715 has been shown to be strongly associated with AMD (Fisher et al., 2005; Maller et al., 2006; Rivera et al., 2005; Schmidt et al., 2006; Seddon et al., 2007), the function of LOC387715 remains unknown. On the other hand, rs11200638 in the promoter of HTRA1, has also been shown to be strongly associated with wet AMD in Caucasian (Yang et al., 2006) and Chinese Hong Kong populations (Dewan et al., 2006). Based on primary functional studies, rs11200638 is the most likely causal variant for AMD at chromosome 10q26. Here we demonstrate rs11200638 confers similar risks to wet AMD in another Chinese cohort. Interestingly, rs11200638 did not show significant association with drusen in the Chinese cohort, as opposed to previously described findings in a Caucasian population (Cameron et al., 2007). rs10490924 and rs11200638 were also shown to be in nearly complete LD with each other as described in the Hong Kong population (Dewan et al., 2006). There have been reports describing differences in the characteristics of drusen in Caucasian and Asian populations, particularly in Chinese populations. Soft confluent drusen, characterized by deposits in the macula, are considered a precursor and a hallmark of advanced AMD in the Caucasian population. In contrast, Wet AMD in Asian patients is frequently associated with much fewer drusen or no drusen. In addition, drusen in Chinese patients are frequently small in caliber (<64 μm) as compared to soft large drusen (>124 μm) in Caucasian patients. It is not clear why in the Chinese population those who carry the HTRA1 risk allele have a distinct wet AMD phenotype compared to that in Caucasians. One possible explanation is that there are other genetic and/or environmental factors corresponding to different underlying pathogenetic mechanisms of choroidal neovascularization. Our results confirm that HTRA1 is a major genetic risk factor for wet AMD in the Chinese population. An understanding of the underlying molecular mechanism will allow important insights into the pathogenesis of AMD as well as the development of new therapies.

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